

REMARKS

Claims 1-23 were pending in the present application prior to entry of the present amendment. By virtue of this response, claims 1-23 have been cancelled, without prejudice or disclaimer, and new claims 24-42 have been added. Accordingly, claims 24-42 are currently under consideration. Amendment and cancellation of certain claims is not to be construed as a dedication to the public of any subject matter of the claims as previously presented. Applicants reserve the right to prosecute any cancelled subject matter in related application.

Support for the new claims can be found throughout the specification as filed. In particular, support for claims 24, 25, 34, 35 and 38 that recite a deletion of all or part of E1 or a deletion of part or all of E3 can be found in the specification at least at page 7, lines 13-24. Support for claims 27, 28, 31 and 32 that recite mammalian and human genes can be found in the specification at least at page 32, lines 6-24. Support for claims 33, and 39-40 that recite bovine adenovirus subgroup I or BAV3 can be found in the specification at least at page 11, lines 9-18. Support for claims 41-42 which recite deletion of part or all of an additional viral gene can be found at least at the paragraph bridging pages 7 and 8.

The specification has been amended to make reference to the SEQ ID numbers of the nucleotide and amino acid sequences disclosed therein. Inasmuch as SEQ ID 1-34 were disclosed in the great grandparent application (U.S.S.N. 08/164,292), to which this present application claims priority, the entry of the amendments to cross reference the SEQ ID NO for each disclosed sequence does not add new matter.

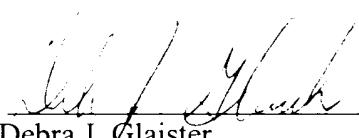
The present application has been amended to correlate the Brief Description of the Drawings with submission of formal figures.

Attached hereto is a marked up version of the changes made to the claims and specification by the current amendment with additions underlined and deletions bracketed. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

CONCLUSION

In the unlikely event that the transmittal letter is separated from this document and/or the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 293102002103. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,


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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning on page 5, containing lines 33-35 and ending on page 6, containing lines 1-13, has been amended as follows:

(Amended)

Transactivation of other viral early genes in permissive infection of human cells is principally mediated by the amino acid sequence encoded in the CR3 region of E1A (Lillie et al., 1986 Cell 46:1043-1051). Conserved cysteine residues in a CysX₂CysX₁₃CysX₂Cys sequence motif (**SEQ ID NO: 30**) in the unique region are associated with metal ion binding activity (Berg, 1986 *supra*) and are essential for transactivation activity (Jelsma et al., 1988 Virology 163:494-502; Culp et al., 1988 PNAS, USA 85:6450-6454). As well, the amino acids in CR3 which are immediately amino (N)-terminal to the metal binding domain have been shown to be important in transcription activation, while those immediately carboxy (C)-terminal to the metal binding domain are important in forming associations with the promoter region (Lillie and Green, 1989 Nature 338:39-44; see Fig. 3).

The paragraph on page 11, containing lines 20-34, has been amended as follows:

(Amended)

Brief Descriptions of the Drawings

[Figure 1] Figure 1A-1L. Sequence and major open reading frames of the left 11% of the BAV3 genome (**SEQ ID NO: 1 through SEQ ID NO: 8**). The region comprises the E1 and protein IX transcription region. The 195 nucleotide inverted terminal repeat sequence identified by Shinagawa et al., 1987 Gene 55:85-93 is shown in *italics*. The amino acid sequence for the largest E1A protein, two E1B proteins and IX are presented. The probable splice donor ([), splice acceptor (]) and intron sequence (*underlined italics*) within the E1A region are marked. A 35 base pair repeat sequence between E1A and E1B is indicated in **bold underline**. Possible transcription promoter **TATA** sequences and possible poly A addition sequences **AATAA** are also indicated.

The paragraph on page 12, containing lines 1-12, has been amended as follows:

(Amended)

[Figure 2] Figure 2A-2B. Regions of homology in the E1A proteins of BAV3 and human adenovirus type 5 (HAd5). The amino acid residue of each serotype is indicated. A. Conserved region 3 (CR3) of HAd5 (**SEQ ID NO: 9**) subdivided into three functional regions as defined by Lillie et al (1989) *Nature* 338:39-44 and described in the Background of the Invention. The intron sequence of BAV3 E1A occurs within the serine amino acid codon at position 204 (**nucleotide positions 1216-1322 of SEQ ID NO: 1**). B. A portion of conserved region 2 (CR2) of HAd5 (**SEQ ID NO: 10**), showing the residues thought to be important in the binding of retinoblastoma protein Rb (Dyson et al., 1990 *J. Virol.* 64:1353-1356), and the comparable sequence from BAV3 (**SEQ ID NO: 34**).

The paragraph on page 12, containing lines 13-16, has been amended as follows:

(Amended)

[Figure 3] Figure 3A-3B. Homology regions between the HAd5 and E1B 19k (176R) protein (**SEQ ID NO: 11 and SEQ ID NO: 12**) and the corresponding BAV3 (157R) protein (**amino acid positions 83-99 and 136-142 of SEQ ID NO: 4**). The amino Acid residue number for each of the viruses is indicated.

The paragraph on page 12, containing lines 17-22, has been amended as follows:

(Amended)

[Figure 4] Figure 4A-4B. The C-terminal 346R of HAd5 E1B 56k (496R) (**SEQ ID NO: 13**) and the corresponding BAV3 protein (420R) (**amino acid positions 74-420 of SEQ ID NO: 6**). The HAd5 protein comparison begins at residue 150 and the BAV3 (in italics) at residue 74. The amino terminal regions of these proteins which are not presented show no significant homology.

The paragraph on page 12, containing lines 23-25, has been amended as follows:

(Amended)

Figure 5. Homology comparison of the amino acid sequence of HAd5 protein IX (**SEQ ID NO: 14**) and the corresponding protein of BAV3 (**SEQ ID NO: 8**) (in italics).

The paragraph on page 13, containing lines 1-8, has been amended as follows:

(Amended)

[Figure 7] Figure 7A-7R. Nucleotide sequence of BAV3 between 77 and 92m.u.

(SEQ ID NO: 15 through SEQ ID NO: 26) showing ORFs that have the potential to encode polypeptides of at least 50 amino acids after the initiating methionine. The nucleotide sequence was analyzed using the program DISPCOD (PC/GENE). Potential N-glycosylation sites (N-X-T/S) and polyadenylation signals are underlined and the first methionine of each ORF is shown in bold.

The paragraph on page 13, containing lines 9-20, has been amended as follows:

(Amended)

[Figure 8] Figure 8A-8C-3. Comparison between the predicted amino acid sequences for the ORFs of BAV3 and known proteins of HAd2 or -5 using the computer program PALIGEN (PC/GENE), with comparison matrix structural-genetic matrix; open gap cost 6; unit gap cost 2. Identical residues are indicated by a colon and similar residues by a dot. (a) Comparison between the predicted amino acid sequence encoded by the 3' end of BAV3 ORF 1 (**SEQ ID NO: 16**) and the HAd2 hexon-associated pVIII precursor (**SEQ ID NO: 27**). (b) Comparison between the ORF 4 (**amino acid positions 34-154 of SEQ ID NO: 22**) and the HAd5 14.7K E3 (**SEQ ID NO: 28**) protein. (c) Comparison between the predicted amino acid sequence encoded by BAV3 ORF 6 (**amino acid positions 8-983 of SEQ ID NO: 26**) and the HAd2 fibre protein (**SEQ ID NO: 29**).

The paragraph at page 15, containing lines 13-34 has been amended, as follows:

(Amended)

[Figure 11] Figure 11A-11B. Southern blot analyses of restriction enzymes digested DNA fragments of the wt BAV3 or recombinant genomes by using a 696 bp XhoI-NcoI fragment from pSM14 (Fig. 9) and a DNA fragment containing the luciferase gene as probes. 100 ng DNA isolated from the mock (lanes 1, 2, 3), BAV3-Luc (3.1) (lanes 4, 5, 6), BAV3-Luc (3.2) (lanes 7, 8, 9) or wt BAV3 (lanes 10, 11 12)-infected MDBK cells were digested with BamHI (lanes 1, 4, 7, 10), EcoRI (lanes 2, 5, 8, 11) or XbaI (lanes 3, 6, 9, 12) and analyzed by

agarose gel electrophoresis. The DNA fragments from the gel were transferred onto a *GeneScreenPlus*™ membrane and hybridized with a 696 bp Xhol-NcoI fragment from pSM14 (Fig. 9) labeled with ^{32}P using Pharmacia Oligolabeling Kit (panel A). Panel B blot represents duplicate samples as in panel A but was probed with a 1716 bp BsmI-SspI fragment containing the luciferase gene (Fig. 9). The sizes of bands visualized following hybridization are shown in kb on the right in panel A and on the left in panel B.

B: BamHI, E: EcoRI, Xb: XbaI, 3.1: BAV3-Luc (3.1), 3.2: BAV3-Luc (3.2) and wt: wild-type BAV3.

The paragraph at page 16, containing lines 21-30 has been amended, as follows:

(Amended)

[Figure 14] Figure 14A-14B. Luciferase expression in the presence of 1- β -D-arabinofuranosyl cytosine (AraC) in MDBK cells-infected with BAV3-Luc. Confluent MDBK cell monolayers in 25 mm multi-well culture plates were infected with A) BAV3-Luc (3.1) or B) BAV3-Luc (3.2) at a m.o.i. of 50 p.f.u. per cell and incubated in the absence or presence of 50 μg AraC per ml of maintenance medium. At indicated time points post-infection, virus-infected cells were harvested and assayed in duplicate for luciferase activity.

The paragraph beginning at page 16, containing lines 31-35 and ending on page 17, containing lines 1-14 has been amended, as follows:

(Amended)

[Figure 15] Figure 15A-15B. Transcription maps of the wt BAV3 and BAV3-Luc genomes in the E3 region. The genome of wt BAV3 between m.u. 77 and 82 is shown which represents the E3 region. The location of Xhol and NcoI sites which were used to make an E3 deletion are shown. (a) The three frames (F1, F2 and F3) representing the open reading frames (ORFs) in the upper strand of the wt BAV3 genome in the E3 region are represented by bars. The shaded portions indicate regions of similarities to pVIII and E3-14.7 kDa proteins of HAd5. The positions of the initiation and termination codons for ORFs likely to code for viral proteins are shown by open and closed triangles, respectively. (b) The predicted ORFs for the upper strand in E3 of the BAV3-Luc genome are shown after a 696 bp Xhol-NcoI E3 deletion replaced by the luciferase gene. The ORFs for pVIII and E3-14.7 kDa proteins are intact. The

transcription map of the wt BAV3 E3 was adapted from the DNA sequence submitted to the GenBank database under accession number D16839.

The paragraph beginning on page 38, containing lines 1-35 and ending on page 39, containing lines 1-14, has been amended as follows:

(Amended)

Example 2 Coding Sequences of the BAV3 E1-Region

BAV3 genomic DNA, from the left end of the genome to the HindIII site at approximately 11%, was cloned into plasmids and sequenced by a combination of manual and automated sequencing. An examination of the resultant BAV3 E1 genomic sequence (Fig 1) revealed a number of interesting features relevant both to transactivation and to other functions associated with adenovirus E1 proteins. On the basis of open reading frames (ORFs) it was possible to assign potential coding regions analogous to those defined in human Ad5 (HAd5). As shown in Fig 1, ORFs corresponding roughly to the first exon and unique region of HAd5 E1A as well as ORFs corresponding to the 19k and 58k proteins of E1B and the ORF corresponding to protein IX were all defined in this sequence. The open reading frame defining the probable E1A coding region begins at the ATG at nt 606 and continues to a probable splice donor site at position 1215. The first consensus splice acceptor site after this is located after nt 1322 and defines an intron of 107 base pairs with an internal consensus splice branching site at position 1292. The putative BAV3 E1A polypeptide encoded by a message corresponding to these splice sites would have 211 amino acids and a unmodified molecular weight of 23,323. The major homology of the protein encoded by this ORF and HAd5 E1A is in the residues corresponding to CR3 (shown in Fig 2). The homology of amino acid sequences on both sides of the putative intron strengthens the assignment of probable splice donor and acceptor sites. The CR3 has been shown to be of prime importance in the transactivation activity of HAd5 E1A gene products. As seen in Fig. 2A the homology of this sequence in the BAV3 protein to the corresponding region of the 289R E1A protein of HAd5 includes complete conservation of the CysX₂CysX₁₃CysX₂Cys sequence motif (**SEQ ID NO: 30**) which defines the metal binding site of this protein (Berg, 1986 Science 232:485-487) as well as conservation of a number of amino acids within this region and within the promoter binding region as defined by Lillie and Green 1989 Nature 338:39-44).

The paragraph beginning on page 41, containing lines 28-35 and ending on page 43, containing lines 1-8, has been amended as follows:

(Amended)

In keeping with the general organization of the E1A region of other adenoviruses, the BAV3 E1A region contains an intron sequence with translation termination codons in all three reading frames and which is therefore probably deleted by splicing from all E1A mRNA transcripts. The largest possible protein produced from the BAV3 E1A region will have 211 amino acid residues and is the equivalent of the 289 amino acid protein translated from the 13s mRNA of HAd5. Two striking features in a comparison of these proteins are the high degree of homology in a region corresponding to CR3 and the absence in BAV3 of most of amino acids corresponding to the second exon of HAd5. In fact the only amino acids encoded in the second exon of BAV3 are, those which are considered to constitute part of CR3. A great deal of work carried out with HAd5 has identified the importance of the CR3 sequences in transactivation of other HAd5 genes. While a detailed analysis of the corresponding BAV3 region and its possible role in transactivation of BAV3 genes needs to be carried out, it is none-the-less interesting to note a couple of possibly pertinent features. The HAd5 CR3 region has been operationally subdivided into three regions (Lillie et al, 1989 *Nature* 338:39-44; see Fig 8); an N-terminal region from 139 to 153 which has four acidic residues and is thought to be important in transcription activation, a central, metal-binding, region defined by the Cys-X₂-Cys-X₁₃-CysX₂-Cys sequence (**SEQ ID NO: 30**) which is essential for both promoter binding and activation, and a C-terminal region (residues 175-189) which is essential for promoter binding. Since, in most instances, E1A protein is thought not to interact directly with DNA (Ferguson et al 1985), the promoter binding regions may be involved in forming associations with proteins which then allow association with DNA. In Fig 2a the BAV3 E1A protein contains the central, metal binding domain and has considerable homology in the carboxy portion of this region. The BAV3 E1A protein also shows identity of sequence with HAd5 in the carboxy 6 amino acids of the promoter binding domain. These features may allow the BAV3 E1A protein to interact with the same transcription activating factors required for HAd5 E1A function. In contrast, except for a Glu-Glu pair there is little homology between the bovine and human viruses in the activation domain. The fact that this domain can be functionally substituted by a heterologous acidic activation sequence

(Lillie et al, 1989 *supra*) suggests that protein specificity is not required in this region and this may allow the BAV3 E1A protein to function in the activation of BAV3 genes. The BAV3 E1A activation region contains six acidic residues in the 18 residues amino to the metal binding domain.

The paragraph on page 44, containing lines 1-26, has been amended as follows:

(Amended)

HAd5 E1B encodes two proteins (19k and 56k) either of which can cooperate with E1A, by pathways which are additive and therefore presumably independent (McLorie et al, 1991 *J. Gen. Virol.* 72:1467-1471), to produce morphological transformation of cells in culture (see for example: Branton et al, 1985 *supra*; Graham, 1984 *supra*). The significance of the conservation of the hydrophobic stretch of amino acids in the central portion of the shorter E1B proteins of HAd5 and BAV3 is not clear as yet. A second short region of homology Gln-Ser-Ser-X-Ser-Thr-Ser (**SEQ ID NO: 31**) at residue 136 near the C-terminus of the BAV3 protein is located near the N-terminus at residue 20 in the HAd5 19k protein. The major difference in both length and sequence of the larger (420R) E1B protein of BAV3 from the corresponding HAd5 protein (496R) is confined to the N-terminus of these proteins. The two proteins show considerable evolutionary homology in the 345 amino acids that extend to their C-termini. A similar degree of homology extends into the N-terminal halves of protein IX of BAV3 and HAd5. Taken together these analyses suggest that while BAV3 and the human adenoviruses have diverged by simple point mutational events in some regions, more dramatic genetic events such as deletion and recombination may have been operating in other regions particularly those defining the junction between E1A and E1B.

The paragraph beginning on page 47, containing lines 28-35 and ending on page 48, containing lines 1-34, has been amended as follows:

(Amended)

HAd2 and HAd5 E3 lies between the pVIII and the fibre genes and encodes at least 10 polypeptides (Cladaras & Wold, 1985, *supra*). The promoter for E3 of these two serotypes lies within the sequences encoding pVIII, about 320 bp 5' of the termination codon. No consensus TATA box is found in the corresponding region of the BAV3 sequences. A non-canonical

polyadenylation signal (ATAAA) for E3 transcripts is located at position 1723, between the end of the putative E3 region and the beginning of ORF 6, encoding the fibre protein, and two consensus signals are located within ORF 6 at positions 2575 and 3565. The polyadenylation signal for the fibre protein is located at nucleotide 4877. Six ORFs were identified in the BAV3 genome between the pVIII and the fibre genes, but only four (ORFs 2, 3, 4 and 5) have the potential to encode polypeptides of at least 50 amino acids after an initiation codon (Fig. 7). The amino acid sequence predicted to be encoded by ORF 2 is 307 residues long and contains eight potential N-glycosylation sites (Fig. 7) as well as a hydrophobic sequence which may be a potential transmembrane domain (PLLFAFVLCTGCAVLLTAFGPSILSGT) (**SEQ ID NO: 32**) between residues 262 and 289. This domain may be a part of the protein homologous to the HAd2 and HAd5 19K E3 glycoprotein (Cladaras & Wold, 1985, *supra*), and the proposed CAd1 22.2K protein (Dragulev et al., 1991, *supra*), but ORF 2 does not show appreciable homology with these proteins. The ORF 4 shows approximately 44% identity with the 14.7K E3 protein of HAd5 (Fig. 6 and 8b), which has been shown to prevent lysis of virus-infected mouse cells by tumour necrosis factor (Gooding, L.R., Elmore, L.W., Tollefson, A.E., Brody, H.A. & Wold, W.S.M. (1988) *Cell*, 53:341-346; Wold, W.S.M. & Gooding, L.R. (1989) *Molecular Biology and Medicine*, 6:433-452). Analysis of the 14.7K protein sequence from HAd2, -3, -5 and -7 has revealed a highly conserved domain, which in HAd5 lies between amino acid residues 41 and 56 (Horton, T.M., Tollefson, A.E., Wold, W.S.M. & Gooding, L.R. (1990) *Journal of Virology*, 64:1250-1255). The corresponding region in the BAV3 ORF 4-encoded protein, between amino acids 70 and 85, contains 11 amino acids identical to those of the HAd5 14.7K protein conserved domain (Fig. 8b).

In the claims

Claims 1-23 have been canceled, without prejudice or disclaimer.

New claims 24-42 have been added.